# Dragonfly Larvae as Bioindicators of Methylmercury Contamination in Aquatic Systems Impacted by Elevated Sulfate Loading

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**Abstract**

This study tested the use of methylmercury (MeHg) in dragonfly larvae as an indicator of MeHg contamination in a range of aquatic systems differing in sulfate loading from mining activities. Dragonfly larvae were chosen due to their ubiquitous distribution in the streams, rivers, and lakes in this study. MeHg concentrations in *Aeshnid*ae dragonfly larvae were positively correlated ($R=0.68$, $p<0.01$) to peak MeHg concentrations in the dissolved phase measured during June and July for both 2012 and 2013. This correlation was stronger in 2012 ($R = 0.866$, $p<0.01$), but showed no correlation in 2013 ($R=0.02$, $p>0.05$). MeHg in dragonfly larvae were not elevated at the highest sulfate sites, but rather the reverse was generally observed**.** MeHg in the water was the best predictor of MeHg in dragonfly larvae leading to the conclusion that MeHg in water from previous studies is a reasonable indicator of MeHg contamination in biota in these systems**.** Record rainfall events in 2012 and above average rainfall in 2013 likely delivered the majority of Hg and MeHg to these systems via interflow. As a result, the impacts of elevated sulfate releases due to mining activities appear to be minimal**.** MeHg concentrations in dragonfly larvae from these systems are similar for both 2012 and 2013 to available data from other studies in the Great Lakes region. Decreasing bioaccumulation factors were observed as DOC increased as observed in other studies, also suggesting that elevated sulfate releases are not enhancing MeHg in the impacted systems.

**Introduction**

MeHg ($CH_3Hg^+$) formed in wetlands, sediments, and other ephemeral anoxic environments poses a problem for the aquatic organisms exposed and humans that consume predator fish from impacted water bodies (Wiener et al. 2003, Scheuhammer et al. 2007). Predatory terrestrial organisms such as spiders and songbirds also have connections to aquatic environments through direct predation and emergent insects (Cristol et al. 2008, Wyman et al. 2011). MeHg is formed primarily by sulfate-reducing bacteria in anoxic environments where Hg (II), sulfate and labile organic carbon are readily available (Gilmour and Henry 1991, Gilmour et al. 1998, Branfireun et al. 1999, Jeremiason et al. 2006). However, the conversion of inorganic Hg (II) to MeHg cannot be predicted by simply measuring Hg (II), sulfate, and organic carbon levels in a particular environment. In order to be methylated, Hg (II) must be able to pass through the bacterial membrane (Gilmour et al. 2011, Graham et al. 2012). Binding of Hg (II) by aquatic ligands impacts bioavailability to bacterial cells, but speciation cannot be determined accurately in most aquatic environments and is further complicated by the physical formation of nanoparticles (Benoit et al. 1999, Aiken et al. 2011, Graham et al. 2013). Sulfate availability is important for continuing to fuel sulfate-reducing bacteria, but reduced sulfide species can bind with Hg(II), reducing bioavailability which can be further diminished by the aggregation of reduced sulfide species with dissolved organic matter (Benoit et al. 1999, Aiken et al. 2011). Furthermore, once MeHg is formed it can be demethylated by UV and visible light and by sulfate-reducing and other bacteria (Sellers et al. 1996, Hammerschmidt and Fitzgerald 2010, Graham et al. 2012)

Due to the complications involved with determining and predicting MeHg concentrations, biological indicators that integrate the complex methylation/demethylation process have been sought (Haraguchi et al. 2000, Evers et al. 2003, Wiener et al. 2006, Carrasco et al. 2008, Hammerschmidt and Fitzgerald

2008). Predator fish are not typically a useful indicator as food web structure and complexity differ markedly between aquatic systems (Wiener et al. 2006). Shorter-lived species such as zooplankton can be useful, but may not integrate over a sufficient period of time and it is time-consuming to separate by size and species and to isolate zooplankton from other aquatic organisms and material (Hall et al. 2009). One-year yellow perch have been used as an indicator in several studies, but the studies are limited to aquatic systems containing perch. Methylating environments such as wetlands are often fishless, and sampling can be challenging and time-consuming (Wiener et al. 2006, Haro et al. 2013). Dragonfly larvae have been collected in multiple studies over the years and recently were used as biosentinels of MeHg in a study encompassing multiple lakes in national parks located in the Great Lakes region (Haro et al. 2013). Dragonflies are ubiquitous in many aquatic systems, live for several years in the larval stage, and are relatively easy to collect with inexpensive equipment. Haro et al. (2013) demonstrated that dragonfly larvae identified by family are useful indicators for MeHg in yellow perch and in the dissolved phase.

In this study we investigated several lake, stream, river, and wetland systems in areas of northeastern Minnesota in in 2012 and 2013 (Table I). The systems varied widely in sulfate concentration in overlying water based on the amount of seepage/discharge the systems received from area mining pits. In 2012, sites on the St. Louis River chosen for dragonfly larvae collection included one located upstream of the mining area (SLR 179), one within the mining district (SLR 132) one a short distance downstream of the mining region (SLR 94) and one further downstream (SLR 33). Several mining impacted sites were chosen and included both stream and lake systems. The Long Lake Creek wetland site (LLC WL) site is just downstream of a wetland on Long Lake Creek which receives seepage and discharge from an open pit taconite mine. The Long Lake Creek (LLC) site is further downstream of the LLC WL site and is located near the confluence with the St. Louis River. Dragonfly larvae were collected from Lake Manganika (MNG) which receives input from another open pit and a stream from the city of Virginia's waste water treatment plant. MNG flows into East Two River (ETR) and larvae were collected on ETR near its confluence with the St. Louis River. Lake McQuade (McQ), a lower sulfate site that flows into West Two River (WTR), receives some water from taconite mines, but is less impacted than Manganika. WTR was also sampled near the confluence with the St. Louis River. One other low sulfate site was chosen on a fork of the West Swan River located at Minnesota Highway 73. This study continued the summer of 2013 with several other main tributaries to the St Louis River being sampled (Table I). Two sites on the St Louis River remained the same in 2013 and ETR and WTR were also sampled both years.

Dragonfly larvae are present in all of the above aquatic systems and were chosen in this study as potential bioindicators of the net methylation/demethylation process in each system. Filtered water samples were also collected from each of the above sites and analyzed for total Hg and MeHg. Ancillary water chemistry measurements including dissolved organic carbon (DOC), major cations and anions, and nutrients. The primary objective of this study was to test the use of *Aeshnidae* larvae as a bioindicator in sulfate impacted systems. We also compared MeHg in biota to other environmental parameters in an effort to better understand MeHg dynamics in these complex systems.

**Methods**

## Field Collection and Identification

Dragonfly larvae were collected with dip nets at 11 sites in the summer and fall of 2012 (Table I). The majority of larvae were collected in October 2012 from sediments and vegetation in shallow areas at each site. A few larvae were also collected from selected sites in June and July 2012. In 2013 dragonfly larvae were collected in the same fashion from 9 tributaries to the St. Louis River and 3 sites along the St. Louis River during August and September. Upon collection, larvae were refrigerated for 12-24 hours to allow them to clear their gut contents. Larvae were then identified and sorted by family (Needham et al. 2000), measured to the nearest mm and frozen until further processing.

## Laboratory Methods

Larvae were lyophilized in 15-mL metal-free polypropylene centrifuge tubes for 24-72 hours (LABCONCO FreeZone6). In 2012, larvae were generally composited by site, family, and length before being ground with a glass stir rod and weighed to the nearest 0.1 mg. In 2013, larvae were processed individually unless mass was determined to be less than 10 mg, in which case larvae would be processed the same as 2012 methods. Composite sample sizes ranged from 5 to 100 mg of dried material and individual samples ranged from 4 to 123 mg. Samples were digested for 12-18 hours in 5-10 mL (exact volume recorded) of 4.6 M nitric acid in a $60^{o}$ C water bath followed by centrifugation at 3000 rpm for 10 minutes (Hammerschmidt and Fitzgerald 2008).

Aliquots of 100 to 200 μL were analyzed for total mercury and MeHg content. Total mercury (THg) was analyzed on a MERX-T Hg Analyzer (Brooks Rand) while MeHg was analyzed by isotope dilution on an Agilent 7700 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) with sample introduction via a MERX-M system (Brooks Rand).

Total Mercury concentrations were determined by standard stannous chloride reduction dual-amalgamation techniques using accepted trace-metal clean methods(Fitzgerald and Gill 1979). Sample aliquots (100 to 200 μL) were added to ~10 mL of MilliQ deionized water and oxidized by the addition of 200 μL of bromine monochloride solution (0.14 M in 12 N HCl; 27 g KBr + 38 g KBrO3 to 2.5 L 12N HCl) for at least 12 hours then neutralized with 4.3 M hydroxylamine hydrochloride (30 g NH2OH.HCl to 100 mL). A saturated solution of the reducing agent stannous chloride (200 μL) was added prior to Hg analysis on a Brooks Rand MERX-T automated mercury analyzer equipped with a total Hg module and a Brooks Rand Model III atomic fluorescence spectrophotometer.

MeHg (MeHg) concentrations were measured using standard ethylation/isotope dilution techniques (Bloom 1989, Hintelmann and Evans 1997). Sample aliquots were spiked with a known amount of isotopically enriched MeHg ($Me^{201}Hg$) and the pH was adjusted to ~4.8 by neutralizing the nitric acid with potassium hydroxide and the addition of sodium acetate buffer. MeHg species were ethylated using sodium tetraethyl borate followed by analysis on the ICP-MS connected to a Brooks Rand MERX-M system.

## Quality Assurance and Quality Control

We measured MeHg and total Hg (THg) in 59 composite samples from 2012 comprising over 300 individual larvae samples. In 2013, 252 samples were analyzed comprising 341 individual larvae. All samples were corrected based on MeHg and THg in method blank samples. Method blanks were run through the same procedure as the actual samples and the mass of MeHg or THg in the method blanks was subtracted from the mass of MeHg or THg in each sample aliquot.

In 2012, the mean MeHg method blank was 0.78±0.14 pg (n=6), which represents an average of 0.84% of MeHg mass in each aliquot and in 2013 blanks were 0.48±0.10 pg (n=15). Blanks for THg were 3.26±1.56 pg (n=6) in 2012 samples and 4.71±1.06 (n=15) pg in 2013, representing less than four percent of THg mass in sample aliquots. To test homogeneity after grinding samples in 2012, several sample replicates or triplicates were analyzed. The sample replicates were only conducted in 2012 on composites greater than 100 mg where portions of the composite were analyzed. These will be referred to as sample replicates. All samples were digested in whole in 2013. Reproducibility was tested by analyzing sample digests multiple times (duplicates and triplicates). These will be referred to as laboratory replicates. For MeHg the relative standard deviation (RSD) of three laboratory triplicates were 2.54%, 1.67%, and 1.48%. Six sample triplicates for MeHg had RSDs of 9.35%, 41.77%, 2.96%, 6.36%, 42.43%, and 1.37%. Four sample duplicates had relative percent differences (RPD) of 42.79%, 3.74%, 4.37%, and 1.94%. The higher sample RSDs and RPDs demonstrate that homogeneity impacts variability of concentrations more than variability due to the analytical method. In the future, compositing of samples is not recommended unless necessary for smaller larvae in order to obtain more than about 5 mg of ground material. For THg, laboratory duplicates were not analyzed. Sample triplicates had RSDs of 3.44%, 48.06%, 7.99%, 4.74%, 39.03%, and 13.92%. The same samples had higher RSDs for THg and MeHg. Four sample duplicates in 2012 were also analyzed for THg and they had RPDs of 14.60%, 13.83%, 5.49%, and 19.68%. In 2013 sample replicates had a mean RPD of 4.60±0.84% (n=16) for MeHg and 9.1±3.3% (n=16) for THg. Finally, National Institutes of Standards certified reference material of lyophilized mussel tissue (NIST 2976 Mussel Tissue) and lobster tissue (TORT-3, National Research Council Canada) was analyzed to assess accuracy. The mussel CRM had an average THg yield of 63.5±4.7 ng/g (n=9) and the certified value is 61.0±3.6 ng/g while the TORT-3 samples averaged 280.1±7.1 (n=4) and the certified value is 292 ± 11 ng/g. For MeHg, the average observed concentration for the mussel tissue was 24.8±2.2 (n=9) and the certified value is 28.1±0.31.

Accuracy of MeHg measurements is assessed daily by analyzing a calibration standard (Brooks-Rand) and the $Me^{201}Hg$ isotope standard (Oak Ridge National Laboratory). The Gustavus Adolphus Environmental Chemistry lab also participated in a laboratory intercomparison study in 2013 and received the highest possible marks for MeHg accuracy (with scores of 3,3, and 3 out of 3) For THg our scores were 2,3, and 3 out of 3 (http://www.brooksrand.com/instrumentmanufacturinghome/ILC2013%20Report.pdf). The Gustavus lab is #45 in the Brooks Rand report.

**Results and Discussion**

We chose Odonates as a biological indicator to assess system sensitivity to MeHg formation and persistence in this study due to their ubiquitous nature in northern MN aquatic systems. The order

*Odanata* includes the suborders *Zygoptera* (damselflies) and *Anispotera* (dragonflies) (Needham et al. 2000). Our collections consisted mostly of dragonflies and we separated them into three families with most samples coming from the *Aeshnidae* (darner) family. Other families included *Gomphidae* (clubtails) and *Libellulidae* (skimmers) which includes *Macromidae* (cruisers) and *Corduliidae* (emeralds) as subfamilies, although some texts do not consider cruisers and emeralds as part of the *Libellulidae* family (Needham et al. 2000). In this study we grouped the larvae as damselfly, aeshnid, gomphid, or macromid. Aeshnids, being the most common, were collected at every site in both 2012 and 2013; MeHg in aeshnids was ultimately used when comparing MeHg concentration in larvae to other environmental parameters.

Dragonfly larvae hatch from eggs typically attached to vegetation or other aquatic solid surface and are laid by emergent dragonflies (Wallace and Anderson 1995). Although it varies by species, dragonfly larvae can have multiple size classes present in a given year. This is due to eggs being hatched over several months during the summer and by overwintering of the larvae that may occur multiple times as dragonflies can live for several years in the larval phase, particularly in colder climates (Wallace and Anderson 1995). Thus, dragonfly larvae collected at any point in time will likely range in size and age (Wallace and Anderson 1995). Larvae tend to live attached to vegetation or burrowed in shallow sediments and they feed on both vertebrates and invertebrates and will accumulate MeHg over time. They molt multiple times and in the final molt period they leave the water body, their body hardens and they become ready for flight (Wallace and Anderson 1995).

Differences by family and size

Our goal was to compare mercury levels in biota between sites. A recent paper by Haro et al. (2013) found that grouping Odonates by family rather than individual species or by sex was sufficient when examining differences in MeHg concentrations between lakes. Haro et al. (2013) did not examine differences in size, but based on other biota, length or age is an important factor (Wiener et al. 2003).

Grouping the larvae by site and by family (Table II) and examining the average MeHg concentration, no clear pattern emerges based on the limited comparisons available. To assess differences between families and sub-orders, analyzing individuals rather than composites would be preferred, which is reflected in the change of processing method for 2013. At some sites, gomphids have a higher average MeHg concentration, while at others aeshnids have higher average concentrations. Note that many of the gomphid and macromid averages are based on one or two individuals. In the future, if comparisons between families are to be made, more larvae should be collected.

Differences in Hg levels due to variability in larval length at each site could only be marginally examined using the available data; Aeshnids were the only family with enough samples to allow for comparisons. Compositing in 2012 made assessing the effect of size difficult as some composites spanned a broad range of lengths. Ultimately, we compared MeHg concentrations in aeshnids that were greater or less than 25 mm in length and found no clear size dependency. A more complete analysis was possible in 2013 and we did not find significant correlations between length and MeHg concentrations. We conclude that variability between sites is greater than variability with length, which is supported by the

conclusions of Haro et al. (2013). Based on this finding, we used a weighted average MeHg concentration in aeshnids to compare between sites for 2012. In 2013, composites were only used when sample mass was insufficient and the compositing had little impact on calculating the mean MeHg concentrations in aeshnids at each site.

MeHg as a percentage of THg

We focused mainly on MeHg concentrations in larvae, but also measured THg. MeHg averaged 72.4 ± 12.8 percent of THg, similar to that found by Haro et al. (2013) and other studies (Kidd et al. 2012). If most mercury in organisms is MeHg, the organism is typically feeding on living biota, while scrapers, grinders, and shredders feeding on detritus and terrestrial debris tend to have a lower portion of mercury in their body as MeHg (Kidd et al. 2012). We did not observe any systematic differences in percent of THg as MeHg, but this could be investigated further.

St Louis River Sites

*Site 1 St Louis River Mile 33 @ Cloquet (2012 and 2013)*

The river is deep and wide at river mile 33 in Cloquet, with poor habitat for dragonfly larvae with little emergent vegetation and harder, rockier substrate near shore. In 2012, a total of seven larvae were collected over two sampling periods (Table II) and all were of the *Aeshnidae* (darner) family. Four samples collected in July 2012 were analyzed individually and 3 individuals collected in October were composited. The larvae from this site were larger on average compared to the other sites. Concentrations of MeHg ranged from 50-150 ng/g dry weight and THg ranged from 93-147 ng/g. The weighted averages were 108 ng/g MeHg and 132 ng/g THg.

The 2013 samples from this site were taken just above the town of Cloquet next to Dunlap Island. The river is narrower on this side of the island, about 40 meters. The St. Louis River in this area has river islands and some small river dams down river from this point. At this site 17 aeshnids, 4 gomphids, and 3 marcromids were collected. *Aeshnidae* MeHg concentrations ranged from 56.4-164.2 ng/g and total mercury concentration ranged 90.4-179.0 ng/g. The averages were 102.6 ±7.7 ng/g MeHg and 137.1 ±7.5 ng/g THg.

*SLR 94 (2012)*

Habitat for dragonfly larvae was not ideal at this site in the fall of 2012 with little emergent or overhanging vegetation. Fortunately, some additional larvae were caught in July when waters were higher. Several *Zygoptera* (damselflies) were sampled in the fall along with some stoneflies and a single *Gomphidae* in the summer. A total of eight *Aeshnidae* (aeshnids) were sampled with 5 individuals (20 to 30 mm in length) analyzed separately in the summer and one composite of three smaller individuals (12 to 15 mm) analyzed in October 2012. Darner MeHg concentrations ranged from 31 to 182 and 46 to 292 ng/g for THg. The weighted averages were 81 ng/g MeHg and 114 ng/g THg. The highest darner MeHg concentration of 182 ng/g was more than double the next highest observed concentration. Removing the high outlier resulted in a weighted mean of 67 ng/g MeHg and 89 ng/g THg.

*SLR 132 (2013)*

Highway 7 in St. Louis County crosses the Forbes location on the St. Louis River 2.5 miles upriver from where East Two Rivers enters the St. Louis River. Banks are heavily vegetated and channel width is close to 35 meters across. A total of 12 aeshnids, 13 gomphids, and nine marcromids were collected. MeHg concentrations ranged from 28.4-213.1 ng/g with an average of 130.0±17.2ng/g and total mercury concentrations ranged from 57.3- 283.8 ng/g with an average of 160.2 ±20.1 ng/g.

*SLR 179*

No collections were made at this site during summer 2012, but a substantial collection with both large and small aeshnids was gathered in the fall. Darner larvae collected in the fall were split into a larger size composite (30 to 38 mm; n=10) and a smaller composite (13 to 18 mm; n=6). A single macromid (15 mm) and gomphid (30 mm) were also analyzed from the fall collection. A size dependency was observed with a higher MeHg (244 ng/g) and THg (288 ng/g) in the bigger larvae compared to the smaller larvae (MeHg = 141 ng/g and THg = 199 ng/g; Table IV). The single darner larvae was similar in concentration to the composite sample of smaller aeshnids. Weighted averages for the aeshnids were 202 ng/g MeHg and 253 ng/g THg. Comparing similar species and sizes, MeHg concentrations in dragonfly larvae are highest at this site upstream of mining activities. Site 1 at Cloquet and Site 2 (Toivola), tended to have similar MeHg levels in dragonfly larvae.

In 2013 A total of 26 dragonfly larvae were collected, 9 aeshnids, 12 gomphids, and 5 marcromids. MeHg concentrations ranged from 36.5-232.2 ng/g with an average of 110.4 ±14.4ng/g and total mercury ranged from 66.5-320.4 ng/g with an average of 148.3 ±14.4ng/g.

<u>Other sites:</u>

*Partridge River (2013)*

Partridge River is 37 miles long and starts 5 miles southwest of the town Babbitt and is impacted by mining discharges. It flows through Hoyt Lakes and enters the St. Louis River 2.5 miles south of Aurora. The collection site is along the county highway 110 which is 2.5 miles from the St. Louis River. Channel length is about 30 meters across and low channel bank walls that are vegetated in grasses. Aquatic plant life grows near the channel banks. South of the bridge, the river runs through an area of fast flowing water over larger rocks that create turbulence. Partridge River had the most dragonfly larvae collected at it with 18 aeshnids, 15 gomphids, and 11 macromid. MeHg concentrations ranged from 13.1-266.8 ng/g with an average of 129.6 ±16.9 ng/g and total mercury concentrations were 33.7-283.4 ng/g with an average of 157.4 ±15.4 ng/g.

*Embarrass River (2013)*

The Embarrass River is 50.5 miles long and starts near Babbitt Minnesota. It flows southwest into the St. Louis River 10 miles upriver from Forbes. The river flows through six lakes, Sabin, Wynne, Embarrass, Cedar Island, Fourth, and Esquagama Lake. The sampling site, next to the Bodas Road Bridge about a mile before the St. Louis River, has a channel width of 14 meters. Here, 26 dragonfly larvae were

collected including 20 aeshnids, five gomphids, and one macromid. MeHg concentrations range from 31.1-221.1 ng/g with an average of 151.8 ±11.2 ng/g and total mercury concentrations range from 44.4-291.2 ng/g with an average of 176.8 ±14.9 ng/g.

*East Two River (2012 and 2013)*

East Two runs parallel to West Two river starting immediately south of the mining area to the west of Virginia Minnesota and has a confluence with the St. Louis River 3.7 miles south of Forbes. A total of 21 aeshnids, one gomphid, and six macromid were collected in 2013. MeHg concentrations ranged from 39.3-154.1 ng/g with an average of 82.6 ±8.5 ng/g and total mercury concentrations were 47.9-179.8 ng/g with an average of 107.2 ±9.2 ng/g.

*West Two River (2012 and 2013)*

West Two run parallel to East Two river starting soon south of the mining area to the west of Virginia Minnesota and has a confluence with the St. Louis River 4 miles south of Forbes. A total of 20 aeshnids, and 12 gomphids (no macromid) were collected. MeHg concentrations ranged 40.4-102.7 ng/g with an average of 64.3 ±4.2 ng/g and total mercury concentrations were 58.6-125.8 ng/g with an average of 85.5 ±5.1 ng/g.

*Swan River (2012 and 2013)*

Swan River starts between the town of Hibbing and West Two River. It flows south into the St. Louis River 2.5 miles upriver of Toivola. Samples were collected from the Swan River in both 2012 and 2013, but at different locations. In 2012, the collections were at a small fork of the river at Highway 73 that is not impacted by sulfate discharges, while in 2013 the samples were collected closer to the confluence of the St. Louis River. A total of eight aeshnids, five gomphids, and one macromid were collected near the confluence of Swan with the St. Louis River. MeHg concentrations were 81.3-555.0 ng/g and total mercury concentrations ranged from 108.3-731.2 ng/g.

*Stony Creek (2013)*

Stony Creek is an 18 mile long meandering creek that flows into the St. Louis River a mile up river from where Swan River enters the St. Louis. The closest town is Toivola 3.5 miles away. The creek is narrow, the banks are heavily vegetated, and in 2013 it stopped flowing shortly after July 10. No gomphids were collected, but seven aeshnids and 13 macromid were taken. MeHg concentrations in aeshnids ranged from 63.6-158.2 ng/g with an average of 88.9 ±12.1 ng/g and total mercury concentrations were 183.0-200.5 ng/g with an average of 146.1 ±18.2 ng/g.

*Whiteface River (2013)*

Whiteface River flows 60 miles starting at the Whiteface Reservoir and entering the St. Louis River 6 miles upstream from the town of Floodwood. Whiteface supplies half the St. Louis's water flow at its point of entry. It is a narrow river which rarely exceeds 50 feet across and the banks are mostly underdeveloped. A total of 18 dragonfly larvae were collected, including nine aeshnids, seven gomphids,

and two macromid. MeHg concentrations in aeshnids ranged from 100.4-152.0 ng/g with an average of 126.4 ±9.5 ng/g and total mercury concentrations ranged from 108.3-233 ng/g with an average of 163.7 ±18.3 ng/g.

*Floodwood River (2013)*

Samples were collected at the highway 73 bridge, one half mile before Floodwood River enters the St. Louis River. The channel is 25 meters across and the banks are steep and vegetated with large trees. The river is 31 miles long and starts near the edge of the watershed in Pancake Lake. A total of 11 aeshnids were collected and eight macromids. MeHg concentrations from this site ranged from 44.1-92.9 ng/g with an average of 65.8 ±5.7 ng/g and the total mercury concentration was 62.9-151.3 ng/g with an average of 97.9 ±10.4 ng/g.

*Cloquet River (2013*

A total of 35 aeshnids, 2 gomphids and 7 macromids were collected at this site. The largest gomphids were collected here. MeHg concentrations from this site ranged from 127.8-460.2 ng/g and total mercury concentrations ranged 146.6-480.8 ng/g. The highest MeHg concentrations and THg concentrations came from the Cloquet River.

*Lake Manganika (2012)*

Lake Manganika is located southwest of the city of Virginia and receives mine discharges from an adjacent pit and flow from the municipal waste water treatment plant. It is a hyper-eutrophic lake with extremely high sulfate concentrations. A composite of 92 small mayflies was analyzed along with four aeshnids in two composites and a composite of five gomphids. All were similar in MeHg (16.8-34.9 ng/g) and THg concentration (33.8-58.6 ng/g). The weighted average of the aeshnids was 40.0 ng/g MeHg.

*McQuade Lake (2012)*

McQuade Lake has lower levels of sulfate compared to Manganika, but is impacted by mine discharges. Dragonfly larvae were collected near the outlet in 2012. A total of 10 aeshnids, 5 damselflies, and two macromids were collected. Four aeshnids were analyzed individually and 6 were composited with MeHg concentrations ranging from 86.2-96.4 ng/g and THg ranging from 127.2 to 142.2 ng/g. The weighted average MeHg concentration in aeshnids was 114.2 ng/g.

*Long Lake Creek Wetland (2012)*

Long Lake Creek Wetland is a ditched wetland located just downstream from a mining pit and dragonfly larvae were collected near its outlet in 2012. A total of 21 aeshnids were analyzed as one composite and had a MeHg concentration of 181.0 ng/g and THg of 223.9 ng/g. Composites of two gomphids and two macromids were also analyzed.

*Long Lake Creek (2012)*

Long Lake Creek was sampled near its confluence with the St Louis River near the Forbes monitoring site. The steeply ditched creek bed provided poor habitat during high flow for dragonfly larvae and only three aeshnids were collected along with 17 damselflies and five gomphids. The aeshnids averaged 150.9 and 206.4 ng/g, respectively, for MeHg and THg.

Comparisons between sites and relationships with environmental variables

In this study, we are testing whether dragonfly larvae are: 1) bioindicators of MeHg contamination and sensitivity of the study systems to mercury methylation; and 2) bioindicators of enhanced MeHg contamination resulting from increased loading of sulfate in these impacted systems. To be useful as a bioindicator of MeHg contamination in a system, odonate MeHg levels would be predictive or indicative of other MeHg burdens in the system such the water, surficial sediment, or other biota. A suitable indicator would also be useful for comparing MeHg across systems. Odonate larvae (Gomphidae) were useful predictors of MeHg in yellow perch and MeHg in the dissolved phase across 17 lakes in the Great Lakes region (Haro et al. 2013). Odonates inhabit benthic areas and feed on invertebrates and vertebrates, generally near the sediment-water interface, accumulating MeHg over time.

*Dissolved MeHg and MeHg in dragonfly larvae*

Observed levels of MeHg in dragonfly larvae integrate the exposure of MeHg over their lifetime (Tremblay and Lucotte 1997, Haro et al. 2013). A single collection of dragonfly larvae integrates exposure over time, while each water sample only represents a point in time. Different time periods for water samples were considered and in the end the best relations were generally found between DF MeHg levels and environmental parameters observed during warmer periods of high flow in 2012 and 2013. The time period corresponds to higher feeding rates and the peak Hg and MeHg water concentrations. Hg and MeHg concentrations earlier in the spring and later in the fall are lower and less variable between systems.

MeHg concentrations in dragonfly larvae were compared to (1) MeHg in filtered water samples, (2) THg in filtered water samples, (3) sulfate concentrations in water, (4) total iron concentration in filtered water samples, and (5) dissolved organic carbon levels.

In 2012, a significant relationship ($R^2 = 0.51$) between MeHg in dragonflies (weighted mean of aeshnid MeHg concentrations) and dissolved MeHg averaged over the entire sampling period (May-Oct) was observed (Figure 1). Considering only dissolved MeHg levels during the months of June and July, the relation with dragonfly MeHg improves ($R^2 = 0.75$; Figure 2). The relation improves even more if the St Louis River sites are excluded ($R^2 = 0.95$; Figure 3). These figures suggest that *Aeshnid* dragonfly larvae are appropriate bioindicators of MeHg concentration in these systems. 2012 was characterized by extremely high flows and other years could be different in terms of which time period correlates MeHg in dragonfly larvae.

In contrast, 2013 data showed no correlation ($R^2 = 0.02$; Figure 4) between dragonfly larvae MeHg and dissolved MeHg over the entire sampling period. After focusing on the months of highest MeHg concentration in the water as in 2012, the correlation was still very weak ($R^2 =0.05$; Figure 5). In 2012,

the St. Louis River sites were outliers while in 2013 other sites were the outliers. Compilation of the two years creates a significant correlation ($R^2$=0.46; Figure 6). 2013 was also an above average flow year and heavy rains occurred throughout the watershed in May and June. The Hibbing airport reported 46.62 cm of rain from May through August in 2012 and 34.40 cm over the same period in 2013.

Our study results do not indicate that systems impacted by mining discharges of sulfate exhibit enhanced levels of MeHg in dragonfly larvae relative to non-impacted sites. A previous study also reported that mining-impacted streams did not exhibit elevated MeHg levels in water relative to non-impacted streams (Berndt and Bavin 2011, Berndt and Bavin 2012). Sites SLR 194 and Swan River are non-impacted 2012 sites, but have the highest MeHg concentrations in dragonfly larvae observed. We can compare results to other studies to examine if MeHg in the dragonfly larvae are enhanced relative to dissolved MeHg concentrations. Enhanced concentrations could be observed in dragonfly larvae if methylation in sediments that dragonfly larvae inhabit is stimulated by additional sulfate from mining discharges. In this case, larvae could be feeding in zones of high MeHg concentrations (the surficial sediment), but this might not be reflected in MeHg levels in the overlying water, which is more typically measured (Berndt and Bavin 2012). 2013 data failed to show a significant difference between half of the sites despite running samples individually. ETR and WTR showed the lowest concentration of MeHg in both dragonflies and the dissolved phase despite both having high impact from mining. MNG L is the site with highest impact and drains into ETR, yet both show decreased levels compared to all other data points.

Haro et al. (2013) report a positive relationship between the mean concentration of MeHg in gomphid larvae and the average MeHg concentration in unfiltered lake water for 17 lakes in the Great Lakes region. The current study differed from the Haro et al. study in that multiple types of aquatic systems were examined, filtered water was used, and water and larvae collections occurred in the same year. Their larvae were collected from 2008-2010 while the surface water samples were collected between 2010 and 2012. Filtered water samples as used in this study are generally preferable to unfiltered samples which can be influenced by particles such as zooplankton or other particulate matter that have high Hg levels but are not homogeneous in a given water body. Nonetheless, most mercury in the water in the Haro et al. study would be predicted (Black et al. 2012) to be bound to dissolved organic matter (DOM) given the range of dissolved organic carbon (DOC) reported (3-13 mg/L).

Bioaccumulation Factors

Based on the above, a reasonable comparison can be made between our study and the Haro et al. (2013) work. Aquatic systems in our study had higher mean MeHg concentrations (June-July) in the dissolved phase ranging from <0.1 ng/L to 1.1 ng/L while the Haro et al study had maximum average concentrations of about 0.25 ng/L. Haro et al. mean larval MeHg concentrations ranged from about 10-100 ng/g dry weight while ours ranged from 30 to 200 ng/g. Examining the slopes of Figure 1-2 and Figure 2 in Haro et al. yields an average bioaccumulation factor (BAF) for dragonfly larvae. Higher BAFs would indicate more MeHg accumulated in larvae relative to the MeHg concentration in water.

$$BAF = \frac{[MeHg] in\ dragonflies}{[MeHg] in\ dissolved\ phase}$$

A slope of $10^{5.4}$ (units are L/kg) is found for Figure 1 and $10^{5.1}$ for Figure 2, while the slope in Figure 2 of Haro et al. is approximately $10^{5.5}$, indicating that MeHg concentrations in dragonfly larvae are not unusually elevated in our systems relative to MeHg concentrations in the water. Tsui and Finlay (2011) report similar BAFs for caddisflies in Minnesota streams. The BAF (slope) decreases in Figure 2 relative to Figure 1 since peak MeHg concentrations from June and July in the dissolved phase were used to construct Figure 2.

Recent studies have concluded that MeHg becomes less bioavailable as DOC concentrations increase (Tsui and Finlay 2011) or increase above a threshold of 8.5 mg/L (Chiasson-Gould et al. 2014, French et al. 2014). All of our study systems had average DOC averages exceeding 8.5 mg/L during June and July and most were well above this threshold. A weak relation was found between BAF and DOC in 2012 (Figure 7) with most of the log BAF values occupying a narrow range from 5.2 to 5.5. An obvious exception was Lake Manganika with a log BAF of 5.8. Manganika, which had the lowest MeHg concentration in dragonfly larvae, had high MeHg in the larvae relative to dissolved MeHg. Being hypereutrophic, Manganika had the majority of MeHg existing in the particulate phase, unlike all of the other systems studied where MeHg is predominantly in the dissolved phase and attached to DOC. The narrow range of BAFs at the other sites indicates that a strong relationship (Figure 2) exists between MeHg in dragonfly larvae and MeHg in the dissolved phase. 2013, on the other hand, had log BAFs distributed between 5.0 and 5.8 (Figure 7) and exhibited no relation between MeHg in dragonfly larvae and MeHg in the dissolved phase. Two of the sites with the highest BAFs were the Cloquet and Embarrass Rivers, both of which have lakes/reservoirs upstream from larvae collection sites. The site with the lowest BAF in 2013 was Stony Creek, a small stream that quit flowing during 2013. Sites in 2012 were also variable in nature. It is unclear why 2012 and 2013 had such different trends between BAF and DOC, but it is clear that the effect of DOC on the BAF must be considered when comparing systems with a large range of DOC concentrations.

*Sulfate and MeHg in dragonfly larvae*

Sulfate-reducing bacteria are known to be the primary mercury methylators in most freshwater systems and addition of sulfate to sulfate-limited systems has increased net MeHg production (Gilmour et al. 1998, Branfireun et al. 1999, Jeremiason et al. 2006, Mitchell et al. 2008). All of these referenced systems were anoxic or seasonally anoxic with low initial levels of sulfate. Salt water marshes and ocean tidal zones with high levels of sulfate are less responsive to sulfate additions and sulfide can inhibit mercury methylation (Gerbig et al. 2011). Some of the sulfate added to an oxic systems such as a river or stream could presumably be reduced when it comes in contact with bottom sediments or passes through hyporheic zones in the river or stream. This could be an area of future research to determine the amount of flow passing through hyporheic zones that results in sulfate reduction in some of the study systems. However, no positive correlation exists between sulfate concentrations and MeHg in dragonfly larvae as shown in Figure 8.

## Conclusions

MeHg concentrations in dragonfly larvae are useful indicators of MeHg contamination in aquatic systems. A significant correlation existed between MeHg concentrations in the larvae and MeHg in the dissolved phase. A complicating factor is a decrease in the bioaccumulation factor of MeHg with increasing levels of DOC which was observed in 2013 and is an active area of investigation. The additional sulfate released from mines in 2012 and 2013 did not have any obvious impacts on MeHg in dragonfly larvae. The BAFS and MeHg concentrations measured were similar to other systems in the region and unimpacted systems in the watershed often had higher MeHg concentrations in larvae compared to heavily impacted systems. As expected, we found no evidence of a positive correlation between sulfate concentration and MeHg in dragonfly larvae. Collection of dragonfly larvae and subsequent analysis of MeHg could be used as a screening and monitoring tool to assess spatial and temporal MeHg levels in aquatic systems. The larvae are ubiquitous in Minnesota aquatic systems, they integrate MeHg contamination over time, they are readily sampled and take less time and effort overall than temporal monitoring of MeHg in the water.

## Acknowledgements

We thank Megan Kelly, Benjamin Van Korff, and Derrick Berndt for assistance with larvae collection. Griffin Reed, Nevada Wendlandt, Amy Christiansen, Russell Kruger, and Will Metcalf processed and assisted with analyzing the larvae. Will Metcalf and Signe Jeremiason assisted with identifying the larvae by family. Alison Agather, Michael Walker, Benjamin Van Korff, Bryan Voigt, Anna Huff, and Nathan Olson processed and analyzed THg and MeHg water samples. Funding was provided by the Minnesota Department of Natural Resources Iron Ore Cooperative Research program, the MN DNR Environmental Cooperative Research program, the National Science Foundation Research award number 0923430, and Gustavus Adolphus College.

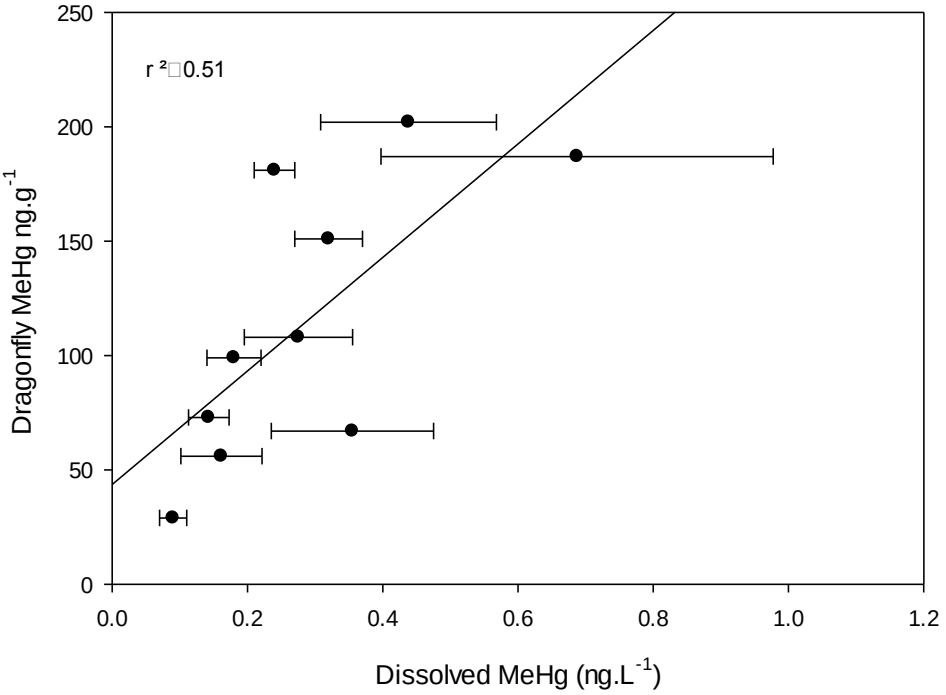


Figure 1. Relation between DF MeHg concentrations and the average dissolved MeHg concentrations measured between May and October 2012. Error bars represent one standard deviation from the mean.

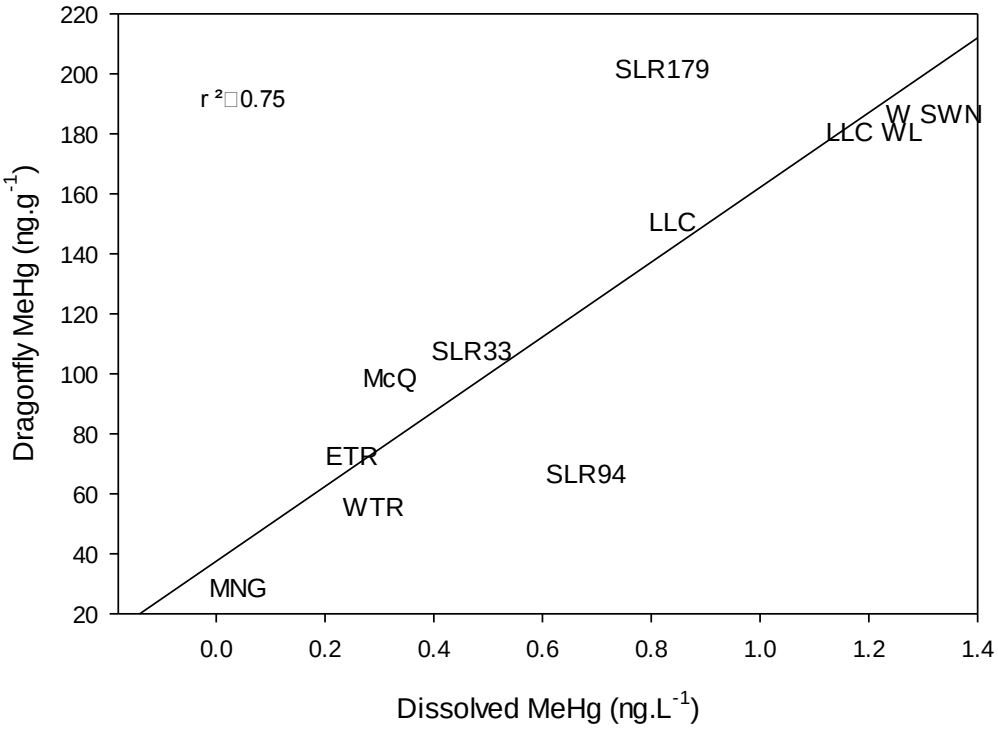


Figure 2. Relation between DF MeHg concentrations and the average dissolved MeHg concentrations measured between June and July 2012, $R^2 = 0.75$.

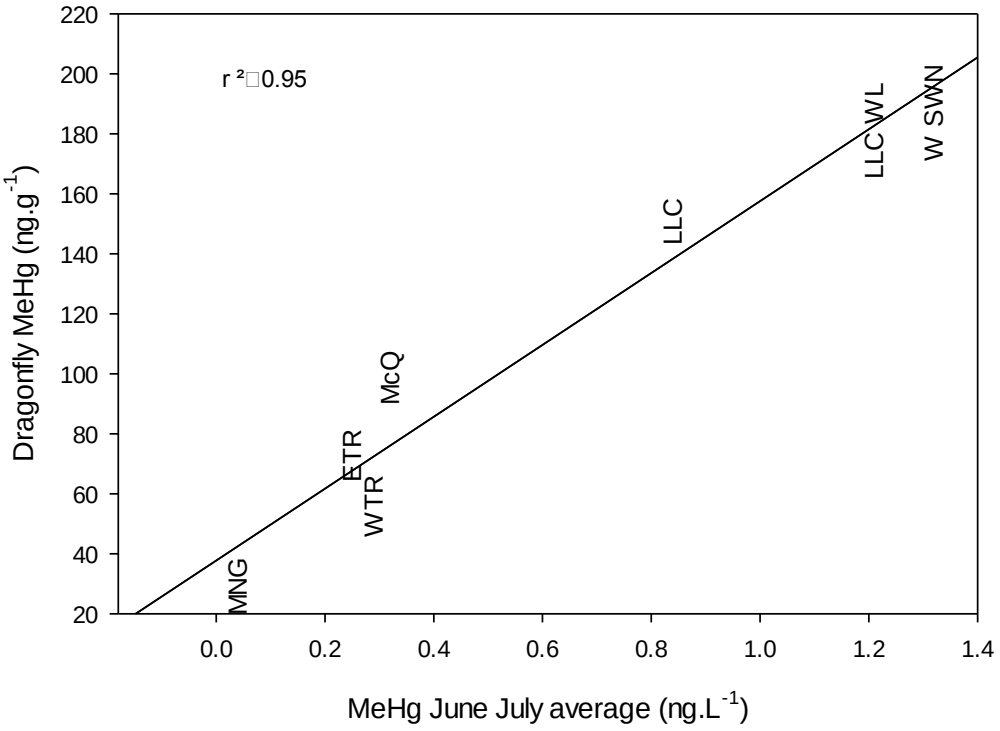


Figure 3. Relation between DF MeHg concentrations (St Louis River sites are excluded) and the average dissolved MeHg concentrations measured between June and July 2012.

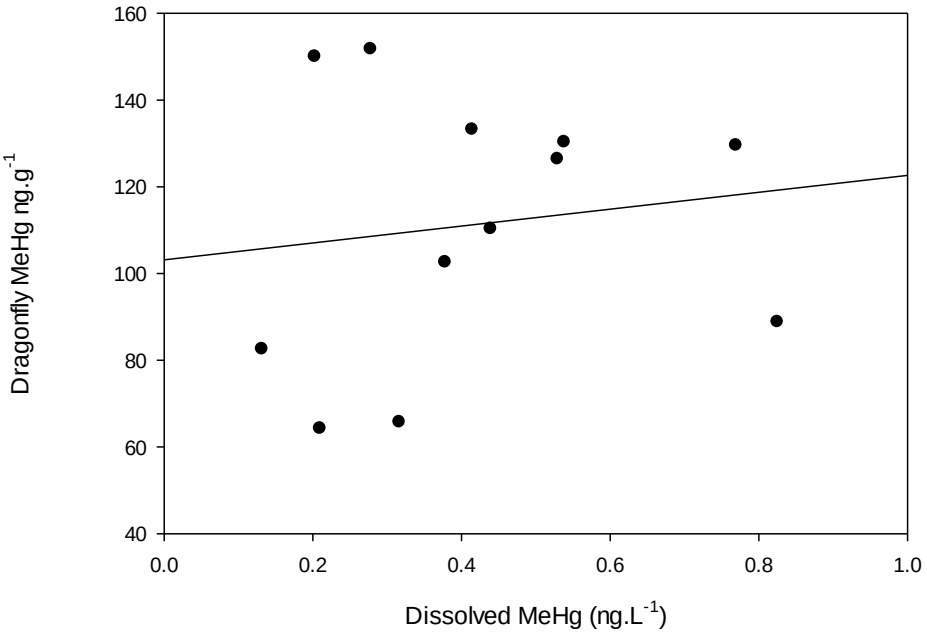


Figure 4. Relation between DF MeHg concentrations and the average dissolved MeHg concentrations measured between June and October 2013. $R^2$= 0.02.

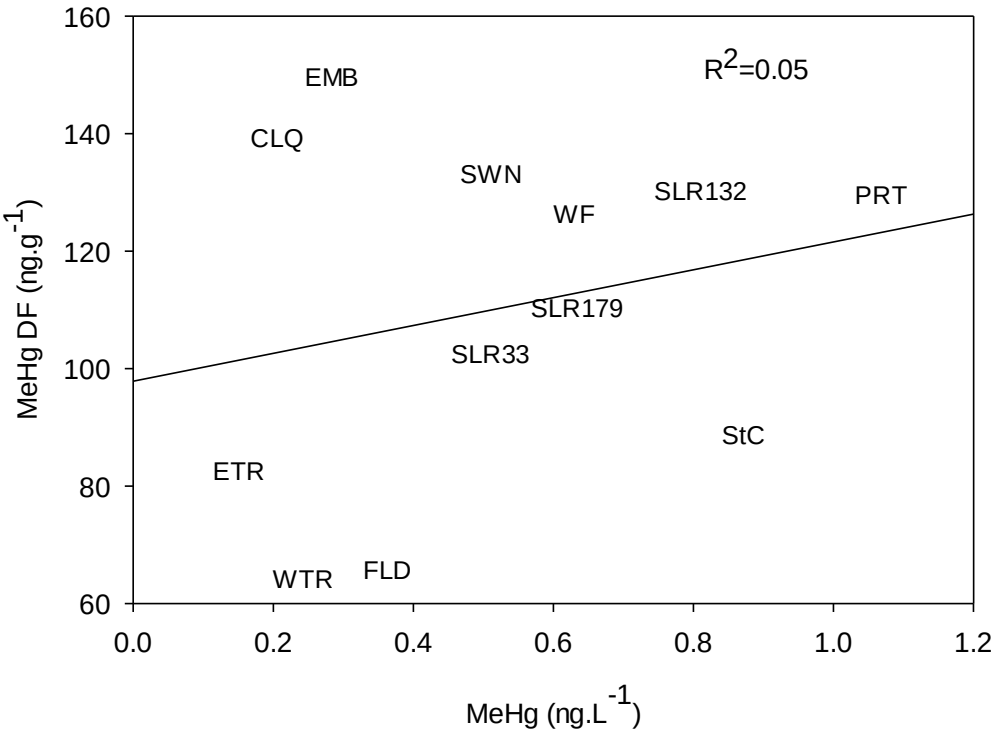


Figure 5. Relation between DF MeHg concentrations and the average dissolved MeHg concentrations measured between June and July 2013.

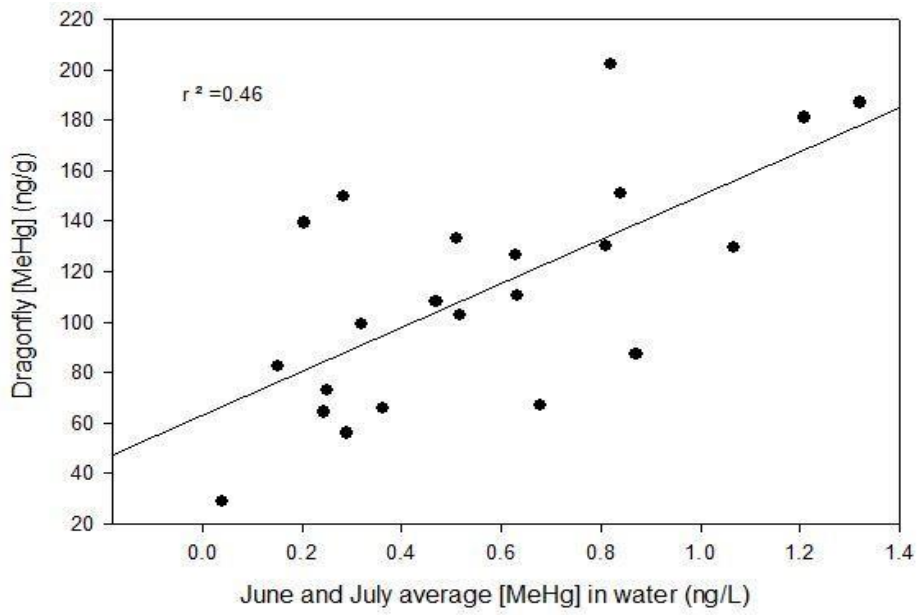


Figure 6. Relation between DF MeHg concentrations and the average dissolved MeHg concentrations measured between June and July for both 2012 and 2013.

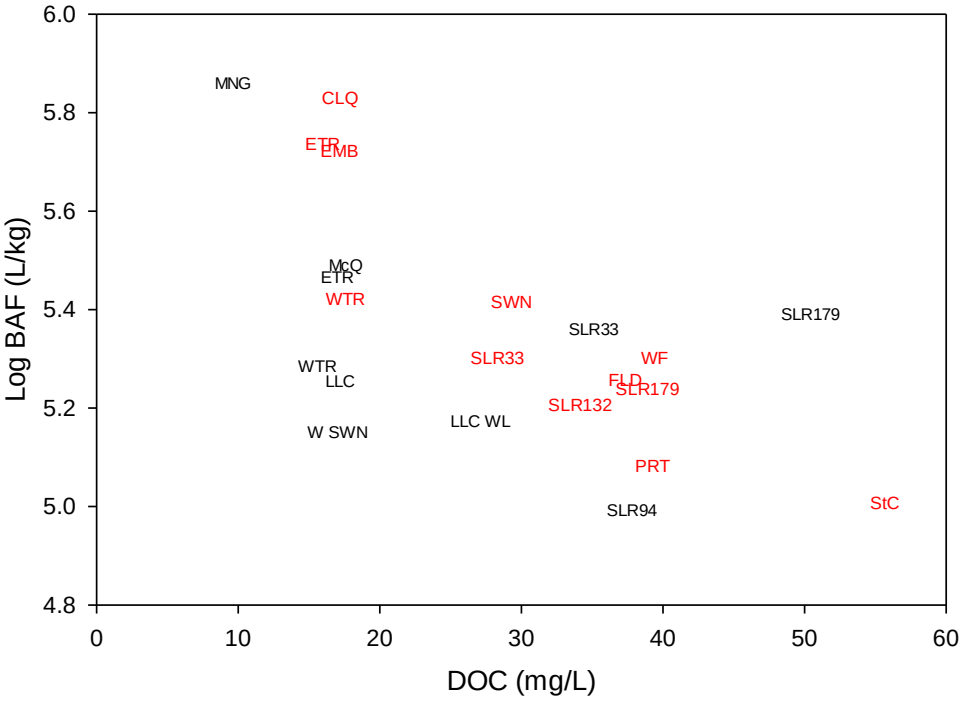


Figure 7. Bioaccumulation factors as a function of DOC. The units for BAF are L over kg of dry mass. 2013 sites are in red and 2012 sites are in black. June-July averages were used for DOC and MeHg concentrations in both years.

Table 1. 2012 and 2013 Sampling Sites.

| Site Abbreviation | Description |
|---|---|
| SLR 33 | St Louis River Mile 33 @ Cloquet (12,13) |
| SLR 94 | St Louis River Mile 94 @Toivola (12) |
| SLR132 | St Louis River Mile 132 @Forbes (13) |
| SLR 179 | St Louis River Mile 179 @ Hoyt Lakes (12,13) |
| LLC WL | Long Lake Creek Wetland Outflow (12) |
| LLC | Long Lake Creek Confluence at SLR |
| MNG | Lake Manganika |
| ETR | East Two River (12,13) |
| WTR | West Two River (12,13) |
| McQ | McQuade Lake (12) |
| W SWN | West Swan River @ Hwy 73 –low sulfate (12) |
| CQT | Cloquet River (13) |
| EMB | Embarrass River (13) |
| FLW | Floodwood River (13) |
| PRT | Partridge River (13) |
| SWN | Swan River-high sulfate (13) |
| StC | Stony Creek (13) |
| WF | Whiteface River (13) |

Table II. Average MeHg concentrations (ng/g) grouped by organism.

| Site | Aeshnid MeHg | Gomphid MeHg | Macromid MeHg | Damsel MeHg | Other |
|---|---|---|---|---|---|
| **SLR33** | 95.4 | | | | |
| **SLR94** | 79.4 | 52.9 | | 34.2 | 57.1[a] |
| **SLR179** | 179.9 | 85.8 | | | |
| **LLC WL** | 181.0 | 75.1 | 100.1 | | |
| **LLC** | 150.9 | 167.9 | | 102.3 | |
| **MNG** | 29.8 | 34.9 | | 13.1 | |
| **ETR** | 143.5 | 163.2 | 69.8 | | |
| **WTR** | 49.6 | 65.0 | | | 114.7[b] |
| **McQ** | 88.2 | | 158.2 | 144.8 | |
| **W SWN** | 221.6 | | | | |
| **2013** | | | | | |
| **CLQ** | 139.4 | 123.8 | 164.2 | | |
| **EMB** | 149.7 | 74.2 | 171.0 | | |
| **ETR** | 82.6 | 66.0 | 87.1 | | |
| **FLW** | 65.8 | | 75.2 | | |
| **SLR132** | 130.4 | 116.3 | 123.9 | | |
| **PRT** | 129.6 | 50.0 | 108.9 | | |
| **SWN** | 133.2 | 93.5 | 132.6 | | |
| **StC** | 88.9 | | 83.0 | | |
| **SLR179** | 110.4 | 68.3 | 145.5 | | |
| **SLR33** | 102.6 | 70.6 | 132.8 | | |
| **WF** | 126.4 | 265.1 | 104.3 | | |
| **WTR** | 64.3 | 52.9 | | | |

[a]composite of 3 stoneflies; [b] one mayfly

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